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The influence of plant growth regulators on flowering, pod set, seed size, and seed yield in soybean

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

NOMENCLATURE

ABA	Abscisic Acid
BA	6-Benzlaminopurine
CYT	Cytokinins
ET	Ethylene
GA	Gibberellins
IAA	Auxins
PGR-A	Plant Growth Regulator A
PGR-B	Plant Growth Regulator B
PGR-C	Plant Growth Regulator C
PGR-D	Plant Growth Regulator D
R1	Beginning Flowering Growth Stage
R3	Beginning Pod Growth Stage
R5	Beginning Seed Growth Stage
R8	Full Maturity Growth Stage
V1	First Trifoliate Growth Stage
V2	Second Trifoliate Growth Stage
V3	Third Trifoliate Growth Stage
V4	Fourth Trifoliate Growth Stage
V5	Fifth Trifoliate Growth Stage
VC	Unrolled Unifoliate Leaves Growth Stage

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ABSTRACT

Soybean (*Glycine max* L.) is a legume crop grown in many countries around the world, including the United States, where it is one of the top two crops in terms of total acres grown. Traditionally, soybean seed has been sold by weight; however, there has been a transition to sale by seed count in the last decade. With seed sold by seed count, soybean seed size has become an important factor in soybean seed production. Soybean seed size is variable and fluctuates largely due to environmental factors. The ability to alter seed size would reduce the cost of producing soybean seed for commercial sale. Further studies may show that growth regulators have the potential to significantly reduce seed production costs. A preliminary field trial was carried out in Glyndon, Minnesota to test the effects of four plant growth regulators on soybean seed size and seed yield in maturity group 0 and 00 soybeans to determine their potential for further study. The objectives of the study were to increase the number of seeds per plant and reduce seed size while maintaining or increasing seed yield.

Multiple treatments were applied that were effective in reducing seed size; however, only one treatment was successful in reducing seed size while still maintaining yield. Treatment PGR-B1 2X resulted in a change of 235.2 seeds per pound when compared to the control plots with no significant change in seed yield. Additional research is recommended with this treatment, as this preliminary study indicates this growth regulator has the potential to achieve this study's objectives.

INTRODUCTION

Soybean *(Glycine max (L.) Merr.)*, is a legume crop grown in many countries around the world. In the U.S., soybean is the second largest field crop with 82.7 million acres grown in 2016 (National Agricultural Statistics Service, 2017). Traditionally, soybean seed has been sold by weight, with 50 pounds of seed equating to one seed unit. Selling seed by weight required farmers to determine the amount of seed needed based on the seed count of the batch they were buying, the desired planting population, and acreage. To simplify the process, seed companies have transitioned to selling by count. In 2010, seed companies began selling soybeans in 140,000 seed units rather than by weight (Moore, 2010). Other companies followed suit and the sale of seed by count has become the industry standard. The switch to selling seed by count made seed size a significant factor in the cost of producing a unit of seed.

Soybean growth and development are determined by the genetics of the variety grown and numerous environmental conditions throughout the growing season. Interactions between the genotype and the environment determine the final yield of a soybean crop in any given season. Seed yield is a product of seed mass, the number of pods per plant, the number of seeds per pod, and the population density. Population density is impacted by the planting rate, germination, and emergence. Seed mass is determined during the seed filling stages with a source-sink relationship existing between the seeds (sink) and the assimilates produced during photosynthesis (source) (Basuchaudhui, 2016). The number of seeds per plant is variable and is influenced most

by environmental factors in the late vegetative and early reproductive stages. Soybean plants commonly abscise a portion of their flowers and pods naturally as the plant adjusts to its growing environment. A soybean plant will produce more flowers than needed and it is common for half of the flowers to abscise during development (Egli, 2005). This process, also known as flower abortion, allows the plant to either produce a greater number of seeds during favorable conditions or to concentrate efforts on the production of fewer seeds during less favorable environmental conditions. Temperature, moisture, photoperiod, soil fertility, and biotic factors are some of the environmental influences impacting rates of flower abortion (Schaik, 1958).

Phytohormones serve as chemical messengers within the plant that play a role in growth and development. These hormones affect source-sink relationships in the plant as they act as signal molecules to coordinate growth and development. Phytohormones are traditionally broken into five different groups including abscisic acid (ABA), auxins (IAA), cytokinins (CYT), ethylene (ET), and gibberellins (GA) (Engels et al., 2012). Additional plant hormones have been identified including jasmonic acid, salicylic acid, brassinosteroids, polyamines, and strigalactones (Engels et al., 2012). Due to their impact on plant growth and development, these hormones are commonly referred to as plant growth regulators (Basuchaudhui, 2016). These growth regulators affect certain aspects of growth and development by promoting, stimulating, delaying, or suppressing plant processes (Engels et al., 2012).

The use of growth regulators to manipulate the growth and development of the plant has the potential to increase flower and pod retention in soybean plants, resulting in

a greater number of seeds per plant. Under normal growing conditions, it is generally accepted that an increase in flower and pod retention will not equate to a significant increase in soybean yield, but will simply result in smaller seeds (Egli, 2005). For seed production purposes, it is necessary that growth regulators would effectively increase seeds per plant without having an impact on seed yield. If growth regulators are successful in only increasing flower and pod retention, average seed size may be lowered due to source-sink relationships within the plant.

With soybean seed production, smaller seed equates to potential cost savings. With more seeds per plant, there are more 140,000 seed units produced per acre, reducing the acreage requirements for the needed supply. Additional cost savings are seen with smaller seed size as transportation and cleaning/conditioning costs are reduced. Smaller seeds reduce the number of trucks needed to transport seed and reduce the overall weight of inbound and outbound seed deliveries due to a reduction in seed mass per 140,000 seed unit. Smaller seeds reduce cleaning/conditioning costs due to a reduction in seed volume resulting in more 140,000 seed units processed per hour. If seed size can successfully be altered, there is potential for significant cost savings for seed production.

Objectives

The overall objective of this study was to test the efficacy of plant growth regulators as a management tool for soybean seed production purposes. A reduction in seed size has the potential to significantly reduce the cost of producing soybean seed for commercial sale. This study was designed to determine if selected growth regulators could be used in commercial seed production as a tool to increase the average number of pods and seeds produced per plant. The second objective of the study was to determine if changes to the number of pods and seeds per plant impact the average seed size of plants treated with selected growth regulators. Lastly, the study measures the effects of these growth regulators on overall seed yield of the genotypes tested to ensure seed yield is maintained or increased.

LITERATURE REVIEW

Seed size is influenced by several factors and as a result, there are numerous studies that investigate the effects of environmental factors and management practices on overall soybean yield and seed size. There are also numerous studies on the effects of growth regulators on soybean, with much of the existing research primarily focused on the effects of growth regulators on seed yield (Vaiyapuri, 2012; Sakar et al, 2002; Nagel, 2001; Takahashi, 1996). Some of these studies have looked at the effects of various growth regulators on the components that make up seed yield including seed mass, the number of pods per plant, the number of seeds per pod, and the population density.

Unfortunately, much of the research does not include sufficient data on the specific effects on seed size because the primary focus of the research was overall seed yield.

Effects of Abscisic Acid (ABA)

The effects of abscisic acid (ABA) application on soybean pod formation and seed yield was studied by Takahashi (1996). Four treatments were applied by foliar spray at three different developmental stages. Applications made early in the reproductive stages (8 days after flowering) promoted soybean pod set (Takahashi, 1996). The data suggest ABA application promoted pod set at early reproductive stages, increased pod fill, and increased seed yield while showing little effect on vegetative growth (Takahashi, 1996). The data from this study suggests abscisic acid has the potential to increase pod set and increase the number of seeds per plant.

Due to insufficient data in this study, we do not know the specific effects of ABA on seed size. Increases in pods per plant were highest with ABA treatments of 1 ppm and 10 ppm; however, these treatments also contributed to even larger increases in grain yields relative to the control. The absence of data regarding the effects of ABA on the number of seeds per pod and resulting total seeds per plant prevents the assessment of the effects of ABA on seed size.

Effect of ABA Application on Filled Pod Number and Grain Yield in Soybean Plants

Treatments:	Filled pod number (no./plant)	% increase over control (%)	Grain yield (g/plant)	% increase over control (%)
ABA (0 ppm)	18.0	-	7.700	-
ABA (1ppm)	21.5*	19.4	9.500*	23.5
ABA (10ppm)	21.0*	16.6	9.345*	21.4
ABA (100ppm)	18.5	2.8	7.352	-4.5

Table 1. Abscisic Acid
Data adapted from Takahashi's (1996) "Table 1. Effect of ABA application on filled pod number and grain yield in soybean plants."
* indicates that the values within the same columns are significantly higher than the control value at P=0.05 by Fisher's LSD.

Effects of Cytokinins (CYT)

A study using 6-Benzlaminopurine (BA), a synthetic cytokinin, was conducted with soybean plants grown both in greenhouse and field trials.

The goal was to evaluate the effects of BA on flower and pod set as well as seed yield. In the greenhouse study, BA was introduced into the plant stem through a wick placed below the first raceme in an attempt to maintain high levels of CYT in the xylem stream for a two-week period during the entire flowering stage (Nagel, 2001). Results of the greenhouse experiments showed BA was effective in increasing the total flowers set and pods per plant, though results did not indicate if increased flower production or reduced rates of flower abortion were the contributing factor (Nagel, 2001). The greenhouse studies produced increased pods, seeds, and seed yield with three of the four BA concentrations (Nagel, 2001). Overall, field trials showed more productive plants than those seen in the greenhouse in terms of seeds and pods produced as well as overall

yield. Results were somewhat inconsistent as treated plants achieved greater numbers of pods, seeds, and seed weight at only one of the four concentrations (Nagel, 2001).

These experiments provide somewhat conflicting data on the effects of CYT as the field trial results do not mirror those of the greenhouse. The desired results of this experiment were achieved in the greenhouse as seed numbers increased along with seed yield. Considering the objectives of this research, the data do not suggest the desired seed production objectives would be achieved through the methods used as a reduction in seed size was not observed.

A three-year study was conducted at the Virginia Polytechnic Institute and State University Horticulture Research Farm at Blacksburg, VA from 1977-1979 to determine the effects of CYT on fruit set and seed development in soybean (Crosby, 1981). Two soybean genotypes were planted with five to seven replications used in the field trials over the three-year period. The first year, five different CYT were applied at the R3 growth stage. The results of the initial testing identified 6-Benzylaminopurine (BA) as the most effective of the CYT, resulting in the second and third year experiments focusing solely on BA treatment compared to the control (Crosby, 1981). Results of the experiment were mixed with one genotype showing a significant increase in seeds per plant and significant increase in total seed weight per plot while the other experienced a significant increase in seeds per plant on one of the two years and no significant increase in total seed weight per plot The study identified genotype, growing environment, and the BA application all as contributing factors influencing the observed seed size (Crosby, 1981).

The results of this experiment provide some valuable information on the effects of CYT on seed size. The field trial data suggests CYT can promote an increase in seeds per plant; however, effects on seed yield varied based on genotype. Significant seed weight differences were not seen with one of the genotypes, but the data suggests a reduction in seed size is not likely with either genotype. For the purposes of this research focus, this data suggests CYT are worth consideration for further research as they play a role in fruit set and seed development.

Effects of Ethylene (ET)

A field experiment was conducted to test the influence of ethylene (ET) supplied via Ethrel coupled with nitrogen application on the growth and yield of soybean (Vaiyapuri, 2012). Data included in the report show the effects on dry matter production, number of seeds per pod, number of pods per plant, seed yield in kilograms per hectare, and stalk and stem yield in kilograms per hectare resulting from foliar application at peak flowering and pod formation (Vaiyapuri, 2012). The focus of the field trials was aimed at increasing soybean yield and as a result provides incomplete data for the type of analysis desired with the objectives of this research. The application of nitrogen in combination with these growth regulators further complicates the data as it adds another variable to the results.

Although no seed size data were included in the report, seed size appears to have been impacted based on comparisons of the average number of seeds per pod, number of pods per plant, and the seed yield (Vaiyapuri, 2012). For example, the 30 kilograms per

hectare of Nitrogen fertilizer and 100 ppm Ethrel treatment data suggest the treatment may have increased seeds per plant significantly while also increasing seed yield. Comparing the number of seeds per plant to the yield data, the data suggests this treatment may be effective in producing a larger number of smaller seeds as would be desired with the current research. Because the focus of this study was on yield and not seed size, the data suggest some interesting results but does not provide the necessary numbers to determine actual seed size from the treated plots.

From 2006 to 2008, field experiments were conducted in Manipur, India. One soybean variety was planted with various treatments applied, including Ethrel at 200 ppm applied at flower-initiation (R1) and pod-initiation (R3) (Devi et al, 2011). The study showed an application of Ethrel at 200 ppm during these stages provided greater vegetative growth with increases in plant height, branching, plant dry weight, and leaf area index (Devi et al, 2011). Yield was also improved using this treatment with increases in pods per plant, seeds per pod, and 100 seed weight (Devi et al, 2011).

Sufficient data were provided for these field trials to allow for numerous conclusions and interpretations. Ethrel application provided increased growth and development across all measured vegetative and reproductive growth characteristics when compared to the control (Devi et al, 2011). Increased pods per plant and seeds per pod were achieved as desired for this research; however, seed size was also increased as seen with the increased 100 seed weights associated with the Ethrel treatment. Based on the results of this study, further research with treatment applications made earlier than

the R1 and R5 stages could be considered to determine if the desired outcomes of this research can be reached.

Plant Characteristics	Growth Regulator	Application at Flower Initiation	Application at Pod Initiation	Both Flower and Pod Initiation	Mean
Plant Height (cm)	Ethrel at 200 ppm	50.60	50.71	50.93	50.75
	Control	37.96	34.71	35.58	36.08
Branches/Plant	Ethrel at 200 ppm	4.3	4.0	4.4	4.2
	Control	2.0	2.0	2.2	2.1
Dry Weight/Plant (g) at 75 DAS	Ethrel at 200 ppm	20.98	21.83	22.59	21.80
	Control	16.21	15.30	13.82	15.11
Leaf Area Index at 75 DAS	Ethrel at 200 ppm	3.30	3.24	3.44	3.33
	Control	2.62	2.50	2.64	2.59
Pods/Plant	Ethrel at 200 ppm	54	55	63	57
	Control	24	24	25	24
100 Seed Weight (g)	Ethrel at 200 ppm	12.32	12.33	12.67	12.44
	Control	10.32	10.48	10.48	10.43
Seeds/Pod	Ethrel at 200 ppm	2.47	2.44	2.65	2.52
	Control	2.20	2.09	2.11	2.13

Table 2. Ethrel Treatment
Data adapted from Devi et al (2011) "Table 1. Effect of bioregulators on vegetative growth parameters of soybean (average for three years)" and "Table 3. Effect of bioregulators on yield components of soybean (average for three years)"

Effects of Gibberellic Acid (GA)

An experiment conducted on the effects of gibberellic acid (GA) on soybean showed an influence on vegetative growth with increases in stem elongation, branching, and leaves per plant when compared to the control (Sakar et al, 2002). Additionally, the investigators found increases in flowers per plant, pods per plant, seeds per plant, and 100-seed weight, when applied 20 and 42 days after planting (Sakar et al, 2002). With

application of GA at 100 and 200 ppm concentration 20 days and 42 days after planting, the number of seeds per plant and average seed weight were increased, resulting in an overall increase in seed yield (Sakar et al, 2002).

This study included data on vegetative and reproductive growth characteristics associated with the application of GA 20 days after planting, 42 days after planting, and both 20 and 42 days after planting (Sakar et al, 2002). For vegetative growth, data indicate significant increases in plant height, number of branches, and number of leaves per plant with both 100 and 200 ppm concentrations of GA. GA applications made 20 days after planting did not result in significantly different measurements than those made 42 days after planting when looking at vegetative growth (Sakar et al, 2002). For reproductive growth, significant increases in number of flowers, pods, fruit set, seeds per plant, seed yield, and 100-seed weight were seen with both the 100 and 200 ppm concentrations when compared to the control (Sakar et al, 2002). Significant differences are seen with reproductive growth between single applications of GA at 20 and 42 days after planting when compared to applications made at both times. Data comparing the effects of GA applied 20 and 42 days after planting show mixed results on reproductive growth though the earlier application shows significantly greater seeds per plant with no significant difference in yield, and 100-seed weight Sakar et al, 2002). With increased yield being the primary focus of the Sakar team's experiment, the desired results were achieved.

The field experiments conducted from 2006 to 2008 in Manipur, India also measured the response of soybean to gibberellin inhibitor Cycocel. One soybean variety

was planted with various treatments applied including Cycocel at 500 ppm applied at flower-initiation (R1) and pod-initiation (R3) (Devi et al, 2011). Although Cycocel reduced plant height, data provided in the study showed greater vegetative growth in terms of branching, dry weight per plant, and leaf area index when compared to the control (Devi et al, 2011). The treatment also stimulated reproductive growth as seen with increases in pods per plant, 100 seed weight of seeds, and the number of seeds per pod compared to the control (Devi et al, 2011).

This study provided ample data to draw conclusions regarding the effects of the gibberellin inhibitor on seeds and pods per plant as well as seed size. The treatment appears to have affected the plant morphology, producing a shorter plant with increased branching and overall vegetative growth. This increase in branching potentially helps to explain the increased pods per plant, which is especially apparent with Cycocel application at flower initiation. The increased pods per plant and seeds per pod seen with Cycocel application align with the desired results of this research. However, increases to 100 seed weight of Cycocel treated plants over the control indicate a positive response to overall yield but a failure to achieve smaller seed. Based on this data, further research could be conducted by altering treatment timing and/or amount in an effort to achieve smaller seeds.

Plant Characteristics	Growth Regulator	Flower Initiation	Pod Initiation	Both Flower and Pod Initiation	Mean
Plant Height (cm)	Cycocel at 500 ppm	32.56	35.67	33.09	33.77
	Control	37.96	34.71	35.58	36.08
Branches/Plant	Cycocel at 500 ppm	3.9	3.8	3.7	3.8
	Control	2.0	2.0	2.2	2.1
Dry Weight/Plant (g) at 75 DAS	Cycocel at 500 ppm	17.07	16.11	16.46	16.55
	Control	16.21	15.30	13.82	15.11
Leaf Area Index at 75 DAS	Cycocel at 500 ppm	3.42	3.34	3.60	3.45
	Control	2.62	2.50	2.64	2.59
Pods/Plant	Cycocel at 500 ppm	40	38	28	35
	Control	24	24	25	24
100 Seed Weight (g)	Cycocel at 500 ppm	11.27	11.19	11.39	11.28
	Control	10.32	10.48	10.48	10.43
Seeds/Pod	Cycocel at 500 ppm	2.55	2.49	2.54	2.53
	Control	2.20	2.09	2.11	2.13

Table 3. Cycocel Treatment
Data adapted from Devi et al (2011) "Table 1. Effect of bioregulators on vegetative growth parameters of soybean (average for three years)" and "Table 3. Effect of bioregulators on yield components of soybean (average for three years)"

The primary focus of soybean growth regulator research has been on seed yield. As a result, data on efforts aimed specifically at a reduction of seed size are limited. For an experiment primarily aimed at reducing seed size for seed production purposes, the hypothesis is that growth regulators can be used to increase the number of seeds produced resulting in a smaller average seed size. In order to conduct this field study, the assistance from a leading company in the seed industry was utilized. With this particular focus being different than previous work, this research will contribute to the existing literature on growth regulators.

MATERIALS AND METHODS

This field experiment was conducted in 2018 as an initial evaluation of the effects of plant growth regulators and treatment timing on four maturity group 00 and 0 soybean varieties grown in Minnesota. The company's Production Research team had previously carried out growth regulator research on longer maturity soybean varieties. The intent of this field trial was to utilize the data from prior studies to determine potential growth regulators for further research with the 00 and 0 maturity group soybeans in this region of the country. This preliminary study looked at treatment combinations and timings to identify potential chemistries for more extensive research.

Field Location

The experiment was performed at a field location in Glyndon, Minnesota. The field location was carefully selected based on soil uniformity. The soybean trials followed corn in crop rotation. Conventional fall tillage treatments representing common practices in the Red River Valley of Minnesota and North Dakota were applied in 2017. Digital mapping of the field occurred in March of 2018, utilizing internal company mapping software to establish GPS plot placement in preparation for planting in the spring. The field was physically marked and flagged prior to planting using a Trimble GPS to ensure plots were accurately placed in their designated locations. An application of Rowel™ preemergent herbicide at a rate of 2.5 ounces per acre took place on May 16th as part of the weed management program.

Variety Selection and Seed Source

The Glyndon study was conducted using four soybean varieties (Variety A, B, C, and D). These varieties were selected to match the field growing conditions and maturity to ensure adequate growing season for obtaining physiological maturity prior to fall frost. These varieties were selected from maturity groups 00 and 0 and ranged in maturity from 0.08 to 0.6. Seed size data was a consideration in variety selection with selected varieties having larger than average seed size within their maturity groups. The selected varieties contained the Roundup Ready 2 Yield® and Roundup Ready 2 Xtend® herbicide trait technologies to allow for the use of glyphosate and dicamba application as part of the weed management program. Seedstock for the experiment passed all commercial seed production guidelines to ensure seed quality and purity. Germination ratings for the seedstock batches used were between 92% and 96% using a 7-day warm germination sand test. The varieties tested and border plot seed were treated with an Acceleron® fully loaded seed treatment which includes fungicide and insecticide. Seed was counted using a Seedburo Seed Totalizer and packaged by variety in preparation for planting.

Growth Regulators

In order to test the effects of growth regulators on soybean, nine different growth regulator treatments were tested in this study. Four growth regulator chemicals were applied (PGR-A, PGR-B, PGR-C, and PGR-D) in the experiment with two being used at multiple rates (PGR-A1/PGR-A2 and PGR-B1/PGR-B2) and two in combination with

one another (PGR-B1/PGR-D, PGR-B2/PGR-D, and PGR-C/PGR-D). The four chemicals used in this study were selected based on prior company research on group two and three maturity soybean varieties. Three of the chemicals (PGR-A, PGR-B, and PGR-C) were selected for application during vegetative growth with the intention of impacting plant flowering. The fourth chemical (PGR-D) was chosen for application during the seed filling stages with the intention of disrupting seed development prior to full maturity. Chemical application rates were based on prior research and were labelled 1X, 2X, and 4X in reference to previous years' application rates. In addition to these nine chemical treatments, untreated plots were included for comparison purposes as the control.

Experimental Design

The field trial was a factorial experiment as the same 10 treatments were applied to four varieties. The trial included four replications per treatment and per variety for a total of 160 plots per location (4 varieties x 10 treatments x 4 replications). Plots were organized as a split plot design with varieties randomly arranged as the main plots and growth regulator treatments as subplots as seen in Figure 2. The field was 22 ranges tall and 8 columns wide with ranges 1 and 22 being border plots to eliminate border effect which can exist on the edges of fields. Each plot contained six rows, with growth regulator application of the middle four rows, and data collection from the middle two rows. This practice was intended to ensure that no contamination of treatments between plots and no neighboring plot effects exist in the data.

Figure 1: Aerial Plot Image (Courtesy of Matthew Wetterling, 2018)

	1	2	3	4	5	6	7	8		
22	Border	Border	Border	Border	Border	Border	Border	Border	22	
21	Treatment D 2X (V3)/Treatment B 1X (R5)	Treatment C 4X (V3)/Treatment B 1X (R5)	Treatment B 1X (R5)	Treatment C 2X (V3)	Control	Treatment D 2X (V3)	Treatment C 4X (V3)	Treatment C 2X (V3)/Treatment B 1X (R5)	21	Set 4
	Variety C	Variety C	Variety C	Variety C	Variety C	Variety C	Variety C	Variety C		
20	Treatment A 2X (V3)	Treatment A 1X (V3)	Treatment A 1X (V3)	Treatment C 4X (V3)	Treatment C 2X (V3)/Treatment B 1X (R5)	Treatment C 4X (V3)/Treatment B 1X (R5)	Treatment D 2X (V3)	Control	20	
	Variety C	Variety C	Variety B	Variety B	Variety B	Variety B	Variety B	Variety B		
19	Treatment A 2X (V3)	Treatment D 2X (V3)/Treatment B 1X (R5)	Control	Treatment A 1X (V3)	Treatment A 2X (V3)	Treatment B 1X (R5)	Treatment D 2X (V3)/Treatment B 1X (R5)	Treatment C 2X (V3)	19	
	Variety A	Variety A	Variety A	Variety A	Variety B	Variety B	Variety B	Variety B		
18	Treatment C 4X (V3)/Treatment B 1X (R5)	Treatment C 2X (V3)	Treatment C 4X (V3)	Treatment D 2X (V3)	Treatment C 2X (V3)/Treatment B 1X (R5)	Treatment B 1X (R5)	Treatment A 2X (V3)	Treatment C 2X (V3)	18	
	Variety A	Variety A	Variety A	Variety A	Variety A	Variety A	Variety D	Variety D		
17	Treatment B 1X (R5)	Treatment D 2X (V3)/Treatment B 1X (R5)	Treatment C 4X (V3)/Treatment B 1X (R5)	Treatment D 2X (V3)	Treatment C 4X (V3)	Treatment A 1X (V3)	Treatment C 2X (V3)/Treatment B 1X (R5)	Control	17	
	Variety D	Variety D	Variety D	Variety D	Variety D	Variety D	Variety D	Variety D		
16	Treatment A 1X (V3)	Treatment C 4X (V3)/Treatment B 1X (R5)	Treatment B 1X (R5)	Treatment D 2X (V3)	Control	Treatment C 2X (V3)	Treatment A 2X (V3)	Treatment C 4X (V3)	16	Set3
	Variety B	Variety B	Variety B	Variety B	Variety B	Variety B	Variety B	Variety B		
15	Treatment C 2X (V3)/Treatment B 1X (R5)	Treatment D 2X (V3)/Treatment B 1X (R5)	Treatment D 2X (V3)/Treatment B 1X (R5)	Treatment B 1X (R5)	Treatment A 1X (V3)	Treatment C 2X (V3)/Treatment B 1X (R5)	Treatment A 2X (V3)	Treatment C 2X (V3)	15	
	Variety B	Variety B	Variety D	Variety D	Variety D	Variety D	Variety D	Variety D		
14	Treatment A 2X (V3)	Treatment C 2X (V3)	Treatment D 2X (V3)	Treatment B 1X (R5)	Treatment D 2X (V3)	Treatment C 4X (V3)/Treatment B 1X (R5)	Treatment C 4X (V3)	Control	14	
	Variety A	Variety A	Variety A	Variety A	Variety D	Variety D	Variety D	Variety D		
13	Treatment C 2X (V3)/Treatment B 1X (R5)	Treatment C 4X (V3)	Treatment D 2X (V3)/Treatment B 1X (R5)	Treatment A 1X (V3)	Control	Treatment C 4X (V3)/Treatment B 1X (R5)	Treatment A 2X (V3)	Treatment C 2X (V3)	13	
	Variety A	Variety A	Variety A	Variety A	Variety A	Variety A	Variety C	Variety C		
12	Treatment A 1X (V3)	Treatment D 2X (V3)/Treatment B 1X (R5)	Treatment C 4X (V3)/Treatment B 1X (R5)	Treatment C 4X (V3)	Treatment B 1X (R5)	Control	Treatment D 2X (V3)	Treatment C 2X (V3)/Treatment B 1X (R5)	12	
	Variety C	Variety C	Variety C	Variety C	Variety C	Variety C	Variety C	Variety C		
11	Treatment C 4X (V3)	Treatment D 2X (V3)	Treatment A 1X (V3)	Treatment A 2X (V3)	Treatment D 2X (V3)/Treatment B 1X (R5)	Treatment C 4X (V3)/Treatment B 1X (R5)	Treatment C 2X (V3)/Treatment B 1X (R5)	Control	11	Set 2
	Variety A	Variety A	Variety A	Variety A	Variety A	Variety A	Variety A	Variety A		
10	Treatment B 1X (R5)	Treatment C 2X (V3)	Treatment A 1X (V3)	Treatment C 4X (V3)/Treatment B 1X (R5)	Treatment D 2X (V3)	Treatment C 4X (V3)	Treatment C 2X (V3)/Treatment B 1X (R5)	Treatment A 2X (V3)	10	
	Variety A	Variety A	Variety D	Variety D	Variety D	Variety D	Variety D	Variety D		
9	3 - Paczol 2x (V3) + Defol 1X (R5)	Treatment C 2X (V3)/Treatment B 1X (R5)	Control	Treatment C 4X (V3)	Control	Treatment C 2X (V3)	Treatment D 2X (V3)/Treatment B 1X (R5)	Treatment B 1X (R5)	9	
	Variety B	Variety B	Variety B	Variety B	Variety D	Variety D	Variety D	Variety D		
8	Treatment C 4X (V3)/Treatment B 1X (R5)	Treatment A 1X (V3)	Treatment D 2X (V3)	Treatment C 2X (V3)	Treatment B 1X (R5)	Treatment A 2X (V3)	Treatment D 2X (V3)	Treatment C 2X (V3)/Treatment B 1X (R5)	8	
	Variety B	Variety B	Variety B	Variety B	Variety B	Variety B	Variety C	Variety C		
7	Control	Treatment C 4X (V3)/Treatment B 1X (R5)	Treatment A 1X (V3)	Treatment A 2X (V3)	Treatment B 1X (R5)	Treatment C 2X (V3)	Treatment C 4X (V3)	Treatment D 2X (V3)/Treatment B 1X (R5)	7	
	Variety C	Variety C	Variety C	Variety C	Variety C	Variety C	Variety C	Variety C		
6	Treatment D 2X (V3)/Treatment B 1X (R5)	Treatment C 2X (V3)/Treatment B 1X (R5)	Treatment C 2X (V3)	Treatment C 4X (V3)/Treatment B 1X (R5)	Treatment A 2X (V3)	Treatment B 1X (R5)	Treatment D 2X (V3)	Control	6	
	Variety B	Variety B	Variety B	Variety B	Variety B	Variety B	Variety B	Variety B		
5	Treatment A 1X (V3)	Treatment C 4X (V3)	Treatment B 1X (R5)	Treatment C 2X (V3)	Treatment C 2X (V3)/Treatment B 1X (R5)	Treatment A 2X (V3)	Treatment C 4X (V3)	Treatment C 4X (V3)/Treatment B 1X (R5)	5	
	Variety B	Variety B	Variety C	Variety C	Variety C	Variety C	Variety C	Variety C		
4	Treatment D 2X (V3)/Treatment B 1X (R5)	Treatment C 4X (V3)	Treatment A 1X (V3)	Treatment C 2X (V3)/Treatment B 1X (R5)	Treatment A 1X (V3)	Treatment D 2X (V3)/Treatment B 1X (R5)	Treatment D 2X (V3)	Control	4	
	Variety D	Variety D	Variety D	Variety D	Variety C	Variety C	Variety C	Variety C		
3	Treatment C 2X (V3)	Treatment A 2X (V3)	Treatment D 2X (V3)	Control	Treatment C 4X (V3)/Treatment B 1X (R5)	Treatment B 1X (R5)	Treatment B 1X (R5)	Treatment C 4X (V3)	3	
	Variety D	Variety D	Variety D	Variety D	Variety D	Variety D	Variety A	Variety A		
2	Treatment C 4X (V3)/Treatment B 1X (R5)	Treatment C 2X (V3)/Treatment B 1X (R5)	Treatment A 1X (V3)	Treatment D 2X (V3)	Treatment C 2X (V3)	Control	Treatment A 2X (V3)	Treatment D 2X (V3)/Treatment B 1X (R5)	2	
	Variety A	Variety A	Variety A	Variety A	Variety A	Variety A	Variety A	Variety A		
1	Border	Border	Border	Border	Border	Border	Border	Border	1	

Figure 2: Plot Layout

Planting

Prior to planting, on May 22 the planter was calibrated according to manufacturer recommendations (full recommendation in Appendix A) to ensure proper plot tripping functionality, which allows for even alleyways and accurate planting population. Planting took place on May 23, 2018. Plots were planted at 140,000 plants per acre with a Seed Research Equipment Solutions plot planter setup for 30-inch row spacing, 17.5 foot long rows, and a 2.5-foot alley between plots as seen in Figure 3. After planting, labeled stakes were placed at the start of each plot to identify individual plots.

Figure 3: VC Growth Stage (Courtesy of Matthew Wetterling, 2018)

Treatment Application

Foliar treatments were applied with a single wheel push sprayer (CO_2) four rows at a time, at a volume of 20 gallons/acre for all treatments with the exception of PGR-B

treatments, which were applied at 30 gallons/acre due to higher rates of the PGR-B chemical. The sprayer used in the study, seen in figure 4, was designed and custom built by members of the Glyndon research team. Calibration of the CO_2 sprayer was performed on June 19th to ensure the proper amount of chemical was applied to the plots (Sandler, 2015). Treatment applications were made based on growth stage with PGR-A, PGR-B, and PGR-C treatments taking place at the third trifoliate stage (V3) and PGR-D treatments at the beginning seed (R5) growth stage (Vaiyapuri, 2012; Devi et al, 2011; Sakar et al, 2002; Nagel, 2001; Takahashi, 1996; Crosby, 1981). Treatment applications of PGR-A and PGR-C were made on June 24th while PGR-B applications took place on June 27th. Applications of PGR-D took place at the R5 growth stage for all varieties. These applications took place on August 7th, 11th, and 18th. In addition to growth regulator applications, glyphosate herbicide was applied on June 19th and July 17th at a rate of 32 ounces per acre as part of the weed management program.

Figure 4: Growth Regulator Application (Courtesy of Matthew Wetterling, 2018)

Treatment	Chemical	Rate	Timing of Application	Treatment Rates:	
1	Control-None	NA	NA	NA	NA
2	PGR-A1	2x	V3	PGR-A1	A oz./acre
3	PGR-A2	4x	V3	PGR-A2	B oz./acre
4**	PGR-B1	2x	V3	PGR-B1	C oz./acre
5**	PGR-B2	4x	V3	PGR-B2	D oz./acre
6**	PGR-C	2x	V3	PGR-C	E oz./acre
7	PGR-D	1X	R5	PGR-D	F oz./acre

Table 4: Treatment Identification and Description
** Additional treatments exist with these chemicals applied in combination with PGR-D

Measurements

Several qualitative and quantitative observations were made with data recorded as numerical counts, measurements, and visual ratings. Data observations were collected throughout the growing season with the following data recorded:

1. Planting Date
2. Final stand count -Taken at the second trifoliate growth stage (V2)
3. Photos/Observations of foliar damage due to treatment – 1, 4, and 7 days post chemical application
4. DOF -Date of Flowering when 50% of plants reach the beginning flowering growth stage (R1)
5. NPODPLNT -Number of pods/plant on 10 consecutive plants at the beginning seed growth stage (R5)
6. PHT -Plant height on 10 consecutive plants at the full maturity growth stage (R8)
7. DOM -Days to physiological maturity taken at the full maturity growth stage (R8)

8. NPODPLNT -Number of pods/plant on 10 consecutive plants at the full maturity growth stage (R8)
9. Damage ratings due to treatment -As needed
10. Yield -Harvest
11. Seed size (seeds/lb.) -Post harvest
12. Germination score of the harvested seed -Post harvest

Figure 5: Plots at V3 Growth Stage (Courtesy of Matthew Wetterling, 2018)

Data were collected throughout the growing season with all observations taken from the two center data rows of each six-row plot. At the second trifoliate stage (V2), stand counts were taken to ensure no emergence issues existed with the plots. Date of

flowering (DOF) notes were taken to determine the number of days from planting to the R1 growth stage of the plots. Observations were made every other day with date of flowering recorded when 50% of the plants in the plot reached the R1 growth stage. Pod count notes (NPODPLNT) were taken at both the R5 and R8 growth stages to compare the affects of the treatments on the mean pod count per plant. Within the middle two data rows of each plot, ten consecutive plants were selected near the center of the row with pod counts recorded on each individual plant.

Figure 6: R5 Pod Count (Courtesy of Matthew Wetterling, 2018)

Wooden stakes were placed at the start and end of these 10 consecutive plants during the R5 counts, as seen in figure 6, to ensure the same plants were counted at the R8 growth

stage. The R5 count was taken to determine the affect of the PGR-A, PGR-B, and PRG-C treatments on pods per plant. The R8 pod count was taken to determine if these treatments had any affect on the retention of pods throughout the reproductive growth stages as well as to determine the affects of the PGR-D treatments on pod count.

Figure 7: R8 Pod Count and Plant Height (Courtesy of Matthew Wetterling, 2018)

Plant height (PHT) notes were taken at the R8 growth stage on the same 10 consecutive plants used for the pod counts and were measured in inches. Maturity notes (DOM) were taken at the R8 growth stage when 95% of the pods had reached full mature color. Throughout the season, photos were taken of foliar damage due to treatment with pictures taken one day, four days, and seven days after application.

Harvest

In order to capture the necessary harvest data, an ALMACO SHP90 combine was used. In preparation for harvest, the combine weigh hopper and moisture systems were calibrated according to manufacturer recommendations (full recommendation in Appendix A) on October 22nd to ensure accuracy of data. Plots were harvested on October 23th with plot weight and moisture measurements taken by the combine. Plot seed was bagged by the combine to provide samples for seed size and germination score data collection. Sample bags were identified by barcode to ensure data quality as seen in Figure 8. After harvest, samples were weighed on a certified scale and counted using a VMEK Metrix analytic lab color sorter (Model MTX 170 36) to determine seed size. Germination scores were determined using a 5-day warm germination test conducted at the company's internal lab.

Figure 8: Harvest Bag (Courtesy of Matthew Wetterling, 2018)

Data Analysis

The data were analyzed by Analysis of Variance (ANOVA) using the SAS® Version 9.4 software developed by the SAS Institute Inc., Cary, North Carolina. An ANOVA test was used to compare treatment means to control means to determine treatment effect on flowering, pod count, seed size, seed yield, date of maturity, plant height, and germination. Treatment means were compared to the control means using the Least Significant Difference and are considered significant at $p \leq 0.05$. The LSD was used as pre-planned comparisons were being made between the treatment means and the control means. The LSD was F-protected.

RESULTS

After carrying out the field trial and data analysis as described in the material and methods, the results were examined to determine the effects of the growth regulator treatments. Significant differences between treatment means and control means existed within each of the measured/observed characteristics. The data indicate the treatments were effective in altering plant growth and development in the tested varieties. Considering the objectives of this study, some of the more significant findings are the affects on seed size and seed yield, where both intended and unintended results are seen in the data.

Flowering

Six of the nine flowering date treatment means are considered significantly different than the control at $p \leq 0.05$. Examining the effects of the PGR-B treatments, significant delays in flowering are seen with all four treatments. This includes the PGR-B1 2X, PGR-B1 2X/PGR-D 1X, PGR-B1 4X, and PGR-B1 4X/PGR-D 1X treatments. Delays associated with these treatments ranged from 17-22.5 days after the mean flowering date of the control plots as shown in Table 5. The application of PGR-A also resulted in significantly different means from the control. The PGR-A1 2X and PGR-A2 4X mean flower dates were 0.8 and 1.1 days earlier than the control mean. The PGR-C treated plots were not significantly different than the control. The application of PGR-D took place at the R5 growth stage, which occurs after flowering. As a result, these treatments did not have any influence on the flowering date of the plots.

Treatments:	*Mean Flowering Date (Days after planting)*
Control	*38.2*
PGR-A1 2X (V3)	*37.4 **
PGR-A2 4X (V3)	*37.1 **
PGR-B1 2X (V3)	*56.1 **
PGR-B1 2X (V3) / PGR-D 1x (R5)	*55.2 **
PGR-B2 4X (V3)	*60.1 **
PGR-B2 4X (V3) / PGR-D 1X (R5)	*60.7 **
PGR-C 2X (V3)	*38.2*
PGR-C 2x (V3) / PGR-D 1X (R5)	*38.1*
PGR-D 1X (R5)	*38.2*

Table 5. Mean Flowering Date
*** Means are significantly different than the control at $p \leq 0.05$.*

Pod Count

At the R5 growth stage, the PGR-B2/PGR-D1X treatment mean was significantly different than the control mean. The mean count for the control plots was 24.7 pods per plant while the mean count for the PRG-B2/PGR-D 1X treated plots was 21.2 pods per plant. Overall, the PGR-B treatments showed considerable variability ranging from the 21.2 pods per plant shown to be significantly lower than the 24.7 pod control mean, to 26.2 with the PGR-B1 2X treatment. PGR-A treated plots had mean counts of 21.9 and 22.1 pods per plant which are not significantly different than the control. PGR-C

treatment counts are both below and above the control mean with the PGR-C 2X mean at 26.1 and the PGR-C 2X/PGR-D 1X mean at 23.1. Lastly, the PGR-D treatment pod counts were not significantly different than the control with a mean of 24.8 pods per plant at the R5 growth stage.

For the pod counts taken at the R8 growth stage, five treatments were significantly different than the control including PGR-A1 2X, PGR-A2 4X, PGR-C 2X/PGR-D 1X, PGR-B1 2X/PGR-D 1X, and PGR-B2 4X/PGR-D 1X. At R8, mean pod numbers in three of the four treatments that included PGR-D were significantly reduced relative to the control. For the PGR-B/PGR-D treatments, the PGR-B2 4X/PGR-D 1X treatment had an 18.0 mean pod count and the PGR-B1 2X/PGR-D 1X treatment had a 20.7 mean pod count. These reductions of 4.1 and 3.6 mean pods per plant are significantly different than the control. The PGR-C 2X/PGR-D 1X treatment was also found to have a significant reduction in mean pod count with 21.0 pods per plant for a reduction of 3.1 pods per plant. Both PGR-A treatments showed reductions in mean pod count that were significantly different than the control mean with both PGR-A1 2X and PGR-A2 4X having 21.3 mean pods per plant for a reduction of 2.8 pods per plant. The remaining treatments, PGR-C 2X, PGR-B1 2X, and PGR-B2 4X, all had mean counts within 1.2 pods per plant of the control mean and were significantly different. Significant interaction ($p \leq 0.05$) were observed between variety and treatment for both the R5 and R8 pod counts with P > F 0.1023 for the R5 counts and P > F 0.0717 for the R8 counts.

Treatments:	*R5 Pod Count Mean*	*R8 Pod Count Mean*
Control	***24.7***	***24.1***
PGR-A1 2X (V3)	***21.9***	***21.3*****
PGR-A2 4X (V3)	***22.1***	***21.3*****
PGR-B1 2X (V3)	***26.2***	***25.2***
PGR-B1 2X (V3) / PGR-D 1x (R5)	***23.1***	***20.7*****
PGR-B2 4X (V3)	***24.0***	***22.9***
PGR-B2 4X (V3) / PGR-D 1X (R5)	***21.2*****	***18.0*****
PGR-C 2X (V3)	***26.1***	***25.3***
PGR-C 2x (V3) / PGR-D 1X (R5)	***23.1***	***21.0*****
PGR-D 1X (R5)	***24.8***	***22.1***

Table 6. Mean Pod Count R5 and R8
*** Means are significantly different than the control at $p \leq 0.05$.*

Date of Maturity

Three of the nine treatments showed no significant affect on maturity as the mean days to full maturity for PGR-A1 2X, PGRA-2 4X, and PGR-B1 2X/PGR-D1X were all within 0.6 days of the control. The other six treatments affected maturity with two causing the plants to mature sooner while the other four delayed maturity. The PGR-C 2X/PGR-D 1X treatment matured at 96.4 days after planting while the PGR-D 1X treatments matured at 94.2 days. Both of these treatments were significantly earlier than the control mean of 100.1 days after planting. Four treatments delayed maturity

including PGR-B1 2X, PGR-B2 4X, PGR-B2 4X/PGR-D 1X, and PGR-C 2X with 106.8, 109.8, 102.6, and 102.6 mean maturity days, respectively, after planting.

Treatments:	***Mean Days to Maturity (Days after planting)***
Control	***100.1***
PGR-A1 2X (V3)	***100.1***
PGR-A2 4X (V3)	***100.7***
PGR-B1 2X (V3)	***106.8*****
PGR-B1 2X (V3) / PGR-D 1X (R5)	***100.3***
PGR-B2 4X (V3)	***109.8*****
PGR-B2 4X (V3) / PGR-D 1X (R5)	***102.6*****
PGR-C 2X (V3)	***102.6*****
PGR-C 2x (V3) / PGR-D 1X (R5)	***96.4*****
PGR-D 1X (R5)	***94.2*****

Table 7. Mean Days to Maturity
*** Means are significantly different than the control at $p \leq 0.05$.*

Seed Size

Eight of the nine treatments were effective in reducing seed size as the mean seeds per pound counts were shown to be significantly greater than the control mean. Looking first at the mean seed size of the control plots, an average of 2,885.9 seeds per pound was observed. The PGR-D 1X treatment showed a significant reduction with a

mean seeds per pound of 4,312.6, for a difference of 1,426.7 seeds per pound. The PGR-B/PGR-D treatments also resulted in significantly different seed size with PGR-B1 2X/PGR-D 1X and PGR-B 4X/PGR-D 1X reducing seed size to 4,147.2 and 4,019.4 seeds per pound respectively. The PGR-C 2X/PGR-D 1X treatment was also significantly different with a reduction of seed size to 4,060.3 seeds per pound. PGR-B1 2X and PGR-B2 4X reduced seed size with differences of roughly 230 seeds per pound from the control, as mean seeds per pound for these treatments were 3,121.1 for the 2X rate and 3,113.9 for the 4X rate. PGR-A was applied at a single (PGR-A1 2X) and double rate (PGR-A2 4X) with both treatments reducing seed size to 3,093.9 and 3,090.3 seeds per pound respectively. The PGR-C 2X treatment did not reduce seed size as the mean seeds per pound was 2,815.8, which is not significantly different than the control mean.

Treatments:	*Seeds Per Pound Mean*
Control	***2885.9***
PGR-A1 2X (V3)	***3093.9****
PGR-A2 4X (V3)	***3090.3****
PGR-B1 2X (V3)	***3121.1****
PGR-B1 2X (V3) / PGR-D 1x (R5)	***4147.2****
PGR-B2 4X (V3)	***3113.9****
PGR-B2 4X (V3) / PGR-D 1X (R5)	***4019.4****
PGR-C 2X (V3)	***2815.8***
PGR-C 2x (V3) / PGR-D 1X (R5)	***4060.3****
PGR-D 1X (R5)	***4312.6****

Table 8. Mean Seeds Per Pound
*** Means are significantly different than the control at $p \leq 0.05$.*

Seed Yield

Of the nine treatments, seven had seed yields that were significantly different than the control. The PGR-B1 2X and PGR-C 2X treatments were not significantly different than the 1,855.9-gram yield mean for the control with yield means of 1,712.4 and 1,854.1 grams respectively. All other treatments were shown to have significant differences in yield from the control. The yield reduction seen with the PGR-A and PGR-B2 4X treatments was roughly 300 grams per plot with 1,555.8 grams for the PGR-A1 2X treatment, 1,524.3 grams for the PGR-A2 4X treatment, and a 1,571.6 gram mean for the PGR-B2 4X treatment. All treatments that included PGR-D at R5 showed

dramatic reductions in yield with these yield means ranging from 291.3 grams to 577.9 grams as shown in Table 8.

Treatments:	***Yield Mean in Grams Per Plot***
Control	***1855.9***
PGR-A1 2X (V3)	***1555.8****
PGR-A2 4X (V3)	***1524.3****
PGR-B1 2X (V3)	***1712.4***
PGR-B1 X (V3) / PGR-D 1x (R5)	***506.1****
PGR-B2 4X (V3)	***1571.6****
PGR-B2 4X (V3) / PGR-D 1X (R5)	***291.3****
PGR-C 2X (V3)	***1854.1***
PGR-C 2x (V3) / PGR-D 1X (R5)	***361.6****
PGR-D 1X (R5)	***577.9****

Table 9. Mean Seed Yield
*** Means are significantly different than the control at $p \leq 0.05$.*

Germination

Considering this research is focused on the use of growth regulators for seed production purposes, the potential affects of these treatments on germination is a necessary measurement to record. Observations of the various treatments show mean germination scores in the 94-96% range. Examining the treatment means, the PGR-B1 2X and PGR-B2 4X treatments are considered significantly different statistically from

the control with both having higher germination rates. Germination data for PGR-B1 2X/ PGR-D 1X, PGR-B2 4X/ PGR-D 1X, PGR-C 2x/ PGR-D 1X, and PGR-D 1X is not complete as there was not a sufficient amount of seed across all varieties to perform the germination testing as indicated in Table 10.

Treatments:	***Germination Mean***
Control	***94.8***
PGR-A1 2X (V3)	***95.3***
PGR-A2 4X (V3)	***95.7***
PGR-B1 2X (V3)	***96.8****
PGR-B1 2X (V3) / PGR-D 1X (R5)	***95.5[1]***
PGR-B2 4X (V3)	***96.8****
PGR-B2 4X (V3) / PGR-D 1X (R5)	***95.4[2]***
PGR-C 2X (V3)	***95.1***
PGR-C 2x (V3) / PGR-D 1X (R5)	***93.3[3]***
PGR-D 1X (R5)	***95.6[1]***

Table 10. Mean Seed Germination
** Means are significantly different than the control at $p \leq 0.05$**.**
[1] No germination data for variety B
[2] No germination data for variety A and B
[3] No germination data for variety B and D

Plant Height

Plant height was recorded at the R8 growth stage on the same 10 consecutive plants used for the R5 and R8 pod counts. A range of mean plant heights from 17.3

inches (PGR-B2 4X/PGR-D 1X) to 23.4 inches (Control and PGR-D 1X) exists across all treatments. The PGR-D 1X and PGR-B1 2X treatments are shown to not be significantly different than the 23.4 inch mean height of the control. All other treatments are considered significantly different than the control with all treatments causing a reduction in plant height. Significant interactions ($p \leq 0.05$) were observed in the analysis between variety and treatment for plant height with P > F 0.3003.

On the day of planting, a Watchdog 2000 series weather station, as seen in Figure 9, was placed in the center of the plot, which collected weather data throughout the growing season up to the date of harvest. Considering the influence of environmental conditions on soybean flowering, pod set, seed size, and seed yield, the weather station data were compared to local historical average high temperatures, low temperatures, and precipitation by month. Comparing the data collected to the historical average, higher temperatures for the month of May and June for the 2018 growing season were observed followed by a relatively average July, and slightly cooler than average temperatures for the remainder of the growing season. Total precipitation recorded at the plot was above average with 15.08 inches recorded from planting to harvest compared to the historical average of 13.42 inches during those same dates. Looking at specific months, June, July, and August all received greater precipitation amounts than the historical average, which is important considering the timing of flowering, pod set, and pod fill.

Figure 9: Weather Station (Courtesy of Matthew Wetterling, 2018)

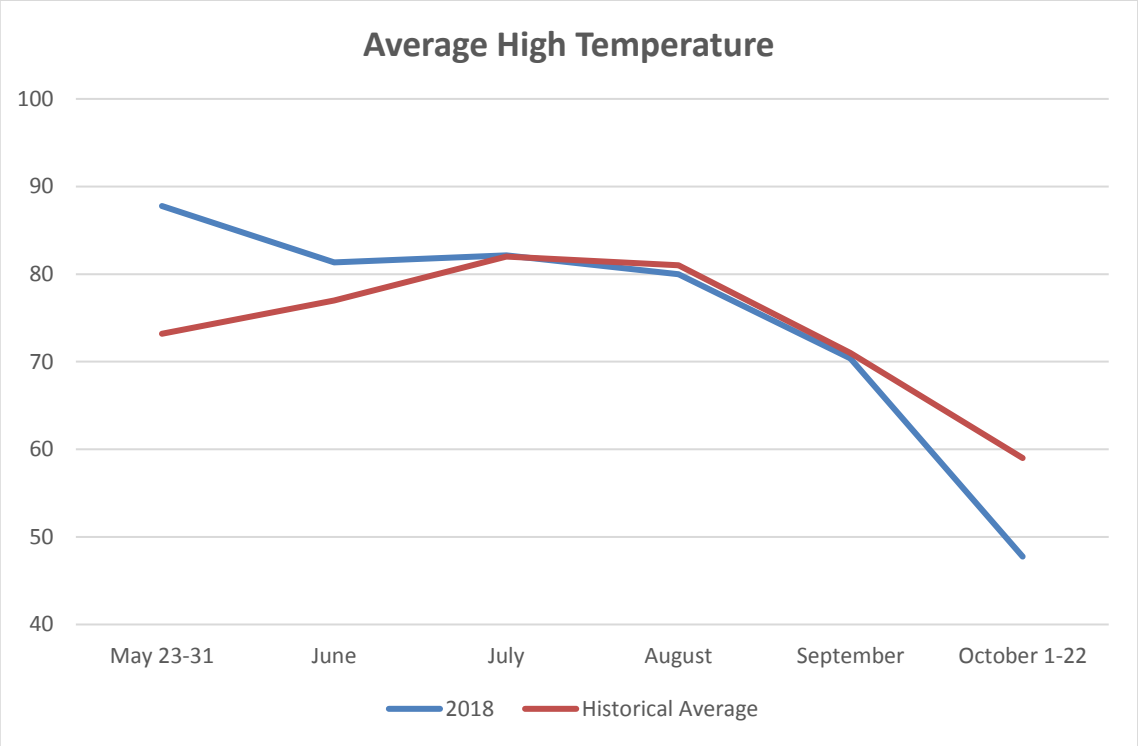

Figure 10. Average High Temperature

Data based on historic average (Weather Underground, 2019) seen in red compared to the weather station data in blue recorded by the Watchdog 2000 Series Weather Station.

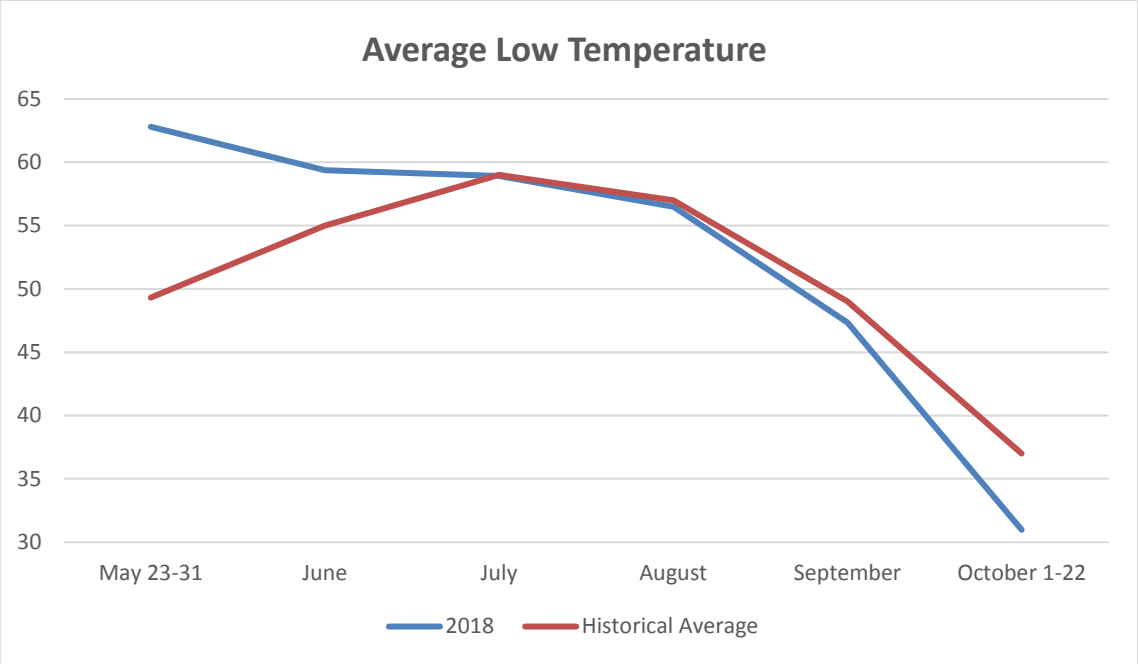

Figure 11. Average Low Temperature

Data based on historic average (Weather Underground, 2019) seen in red compared to the weather station data in blue recorded by the Watchdog 2000 Series Weather Station.

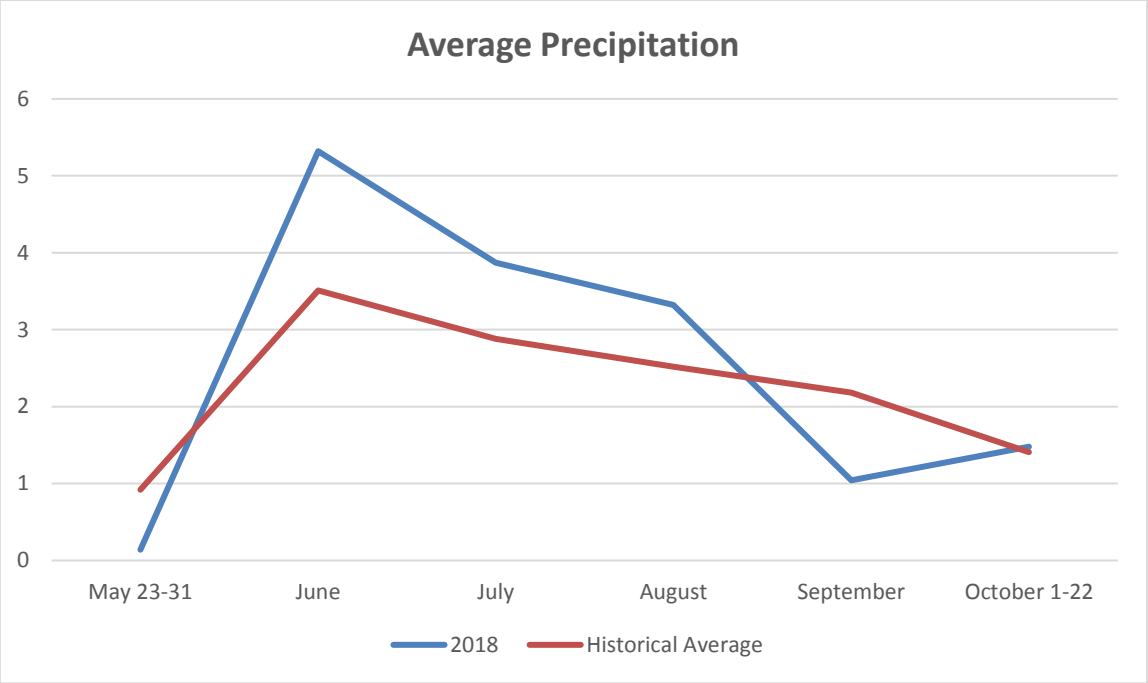

Figure 12. Average Precipitation

Data based on historic average (Weather Underground, 2019) seen in red compared to the weather station data in blue recorded by the Watchdog 2000 Series Weather Station.

DISCUSSION

The objective of this research was to determine the efficacy of plant growth regulators as a management tool to increase seeds per plant, reduce seed size, and to maintain or increase seed yield for soybean seed production purposes. In reviewing the data collected throughout the study, the selected growth regulators achieved varying degrees of influence on the observed characteristics of treated plants. Considering seed growth takes place during reproductive stages, changes to these growth stages can have an affect on seed characteristics. Reviewing the results of the ANOVA, significant findings exist in the flowering date, date of maturity, R5 and R8 pod counts, seed yield, and seed size data.

All nine chemical treatments affected the timing of the reproductive stages. Three of the treatments affected only the days to flowering, three treatments affected only the days to maturity, and three treatments affected both flowering and days to maturity. The most significant findings seen with the flowering data exist with the PGR-B treatments. The PGR-B treatments were made with the expectation that flowering would be impacted. The substantial delays seen with PGR-B were not expected based on what was previously observed in prior research. There are numerous implications associated with the delays in flowering caused by the PGR-B treatments. With these plants flowering 17.0-21.9 days later in the season, they may do so under different environmental conditions that can have an impact on flowering processes. Additionally, delays continue to have an effect on the plants as subsequent reproductive growth stages

are delayed throughout the season. PGR-B treated plots reached full maturity later in the season, resulting in a later harvest date. Lastly, the duration of the reproductive growth stages is affected as greater delays were seen at flowering than at full maturity with the PGR-B treatments, affecting seed growth and development due to shorter reproductive growth stages for these plants.

Another important finding in regard to the timing of the reproductive stages was the impact seen with the PGR-D treatments. PGR-D was shown to reduce the mean days to maturity when applied on its own as well as in combination with the other growth regulators. This application, made during key seed development stages, impacted pod filling and sped up maturation. PGR-D was applied at R5 with the intention of distrupting seed development prior to full maturity, the effect seen on plant maturity were expected and aligned with prior years' observations. The implications of the PGR-D treatment's affect on maturity include less time for seed development, which can have an impact on seed quality and an earlier harvest date.

The most important findings with the pod count data include the affects of the PGR-B treatments observed in the R5 counts as well as the effects of the PGR-D treatments observed during the R8 counts. At the R5 growth stage, the PGR-B2 4X/PGR-D 1X treatment caused a reduction in pod count. There was a large degree of variability seen in the R5 pod count data with the PGR-B treatment counts ranging from 21.2 to 26.2 pods per plant. When the R5 counts were taken, the PGR-D treatments had not yet been made. Considering this, both PGR-B2 treatments (PGR-B2 4X and PGR-B2 4X/PGR-D 1X) had undergone the same treatment up until that point. Despite the

fact that the PGR-B2 4X treatment and the PGR-B2 4X/PGR-D 1X plots had received the same treatment at that time, the PGR-B2 4X pod counts were not significantly different than the control while the PGR-B2 4X/PGR-D 1X were significantly different. The delayed flowering seen with the PGR-B treatments may be associated with the variability seen with the R5 counts. With PGR-A, PGR-B, and PGR-C treatments made during vegetative growth with the intention of impacting reproductive processes, the expectation was that average pod count would be impacted. Pod count data were not as expected as only one treatment significantly changed counts, with the response observed being the opposite of what was intended.

Pod count data were once again taken at the R8 growth stage following the PGR-D treatment applications to determine pod retention from the R5 to R8 growth stage as well as to the see the effects of the PGR-D treatment. At the R8 growth stage, five of the treatments had mean pod counts significantly different than the control mean. The most important findings at the R8 growth stage were the affects of the PGR-D treatment, which resulted in a significant reduction in pods in all but one of the PGR-D treatments. Interestingly, the treatment of PGR-D 1X did not produce mean pod counts significantly different than the control. The PGR-D chemistry produced reductions when used in combination with all other treatments, but failed to do so when used on its own as mean counts were 22.1 pods per plant. Considering PGR-D was applied at R5 with the intention of disrupting seed development, an impact on pod count was anticipated; however, the loss of pods was greater than expected based on what was observed in prior research. Overall, the implications of reduced pod count are a reduction in overall seeds,

potentially larger seed size, and reduced yield. The treatments were unsuccessful in increasing pod counts as intended for this study. As discussed in the results, significant interactions were observed in the analysis between variety and treatment with both the R5 and R8 pod counts (P > F 0.1023 and F 0.0717, respectively). These interactions may be the result of treatment application timing and the maturity differences of the four varieties at the time of application. The interactions while significant statistically, did not appear to be important from a practical perspective.

Although no data were collected on the number of seeds per pod, it is also important to note that visual observations were made on the plots throughout the entire study. From these observations, it was determined that many of the PGR-D treated plots had fewer seeds than non- PGR-D treated plots with some pods containing no seeds. These observations indicate that PGR-D treatments reduced the total number of seeds per plant, which also aligns with the seed yields seen with the PGR-D treatments.

Considering the influence of the plant growth regulators on the flowering and pod counts of the plants, it is not surprising to see considerable differences in seed size between the various treatments. Overall, some of the treatments were successful in reducing seed size within the desirable range for seed production while others over-performed, resulting in seeds too small for seed production. Seed smaller than 4,000 seeds per pound would fall outside of seed production guidelines and would not be useable. The most significant findings are seen with seed size reductions associated with the PGR-A1 2X, PGR-A2 4X, PGR-B1 2X, and PGR-B2 4X treatments, all having seed size within the desirable range for seed production purposes as seed counts ranged from

3,090.3 to 3,121.1 seeds per pound. Another important finding with this data was with the PGR-D treatments, which all resulted in greater reductions than intended, as the seed were too small for seed production purposes. PGR-D also had a negative impact on seed quality as plants receiving these treatments produced a higher percentage of abnormally shaped, wrinkled, and green seeds when compared to the control plot samples. As discussed in the introduction, the implications of reduced seed size for seed production include significant cost saving per unit of seed produced.

Achieving a reduction in seed size was the main focus of this research; however, maintaining or increasing seed yield was also a primary objective as well. Analyzing the seed yield data, many of the treatments that were effective in reducing seed size also showed a reduction in seed yield. The most significant finding was with the PGR-B1 2X treatment, which did not show a significant difference in yield, while reducing seed size with a mean seeds per pound of 3,121.1 compared to the 2,885.9 mean seeds per pound of the control. All other treatments failed to meet the objectives of the study as they did not achieve the desired reduction in seed size or saw a significant reduction in yield.

Seed yield is an important consideration with this study as the potential savings seen with reduced seed size are only realized if seed yields are maintained or improved. Although the PGR-B1 2X treatment was effective in meeting the objectives of the study, there are some potential concerns associated with the delayed flowering date as discussed previously. Further research is needed to determine if this treatment is a good option for meeting the desired objectives of this study. It is important to note that the conclusions made from this study are based on one year of data collected at one field

trial location and longitudinal research across various field locations will be needed to provide more reliable conclusions.

The affects of the treatments on plant height are not a concern considering the objectives of the research; however, plant height data help researchers better understand the impact of the treatments on plant growth characteristics. Plant heights ranged from 17.3 inches to 23.4 inches, with all plots of sufficient height for harvest. The PGR-D 1X and PGR-B1 2X treated plots are not significantly different than the control mean. All treatments, with the exception of PGR-D 1X and PGR-B1 2X, were considered significantly different than the control with treatments reducing plant heights. The affects of these treatments on branching was not determined in this study as no data were collected on the number of branches per plant. Although treatments that reduced height may have resulted in increased branching, differences were not substantial from a practical point of view as any increased branching that may have existed did not result in significant increases in pod count. Significant interactions were observed in the analysis between variety and treatment for plant height, although closer examination of the data did not reveal specific differences in response between the main affects.

There are limitations to consider when examining the data included in this study. These limitations include a delay with the PGR-B application at the V3 growth stage as well as partial germination data seen with plots treated with PGR-D at the R5 growth stage. The PGR-B application was intended to coincide with the PGR-A and PGR-C applications made on June 24th; however, due to delays with chemical availability and wind conditions, the PGR-B application was made on June 27th.

The affects of this delay in application are two-fold. The application of PGR-A, PGR-B, and PGR-C were all intended to take place at the V3 growth stage and with the delays associated with PRG-B, the PGR-A and PGR-C treatments were delayed later into the V3 growth stage. This resulted in application of PGR-A and PGR-C taking place later in the V3 growth stage and closer to flowering than what would have been seen had the PGR-B chemical been available. The decision was made to proceed with the PGR-A and PGR-C applications to ensure treatments took place prior to flowering. When the PGR-B application was made on June 27th, variety A and B had progressed to the beginning flowering growth stage and were no longer at the V3 growth stage as originally planned in the protocol. Variety C and D were still at the V3 growth stage during this application. It is also important to note, prior research data that were considered when determining application timing was collected on group two and three soybeans, which have a longer growing season and generally will progress through additional vegetative stages (V4 and V5) prior to flowering. With earlier maturing varieties, treatments at the V3 growth stage takes place much closer to the start of the reproductive stages.

Although PGR-B treated plots were delayed across all varieties, the delay in flowering with Variety A and B may be connected to this delayed application. Application of PGR-B at the R1 stage with Variety A and B resulted in the loss of the existing flowers and a delayed bloom a few weeks later. Flowers on plants treated with PGR-B did not bloom normally as seen with the control plots as flowers failed to fully bloom. This affect on flowering may have influenced the DOF notes taken on these

plots. As discussed previously, the progress of flowering of the plant greatly influences the number of pods and ultimately seeds the plant produces. Considering the affects of environmental conditions on flower abortion, treatment affect on flowering date is an important consideration. Delays in flowering might result in plants going through this reproductive stage under less desirable environmental conditions and also may reduce the duration of reproductive stages.

All treatments that included the application of PGR-D at the R5 growth stage had incomplete germination data due to insufficient plot seed for germination testing. Germination data for PGR-B1 2X/PGR-D 1X did not include ratings for variety B. PGR-B2 4X/PGR-D 1X did not include data for variety A and B. PGR-C 2X/PGR-D 1X did not include germination data for variety B and D. Germination data for PGR-D 1X did not include rating for variety B. As a result of this limitation, germination data for PGR B1 2X/PGR-D 1X, PGR-B2 4X/PGR-D 1X, PGR-C 2X/PGR-D 1X, and PGR-D 1X should be considered as partial data.

The weather data recorded at the Glyndon plot are an important consideration as weather can have a significant effect on plant growth and development. Moisture and temperature are major factors in plant growth and have an impact on both vegetative and reproductive growth stages. There is a strong relationship between temperature and moisture, as increased temperatures result in greater evapotranspiration rates. Moisture has been shown to effect vegetative growth with reduced leaves, branches, plant height, and dry matter production occurring under moisture stress conditions (Mustapha et al., 2014). Comparing the weather station data to the historical average, precipitation was

above average during the soybean vegetative stages. Temperatures recorded at the plot were above historical averages from planting through the vegetative growth stages, but no moisture stress was observed during this time. Moisture stress during reproductive stages can shorten the duration of the flowering stages, increase flower and pod abortion, reduce seeds per pod, reduce seed size, and reduce seed yield (Staton, 2018). Precipitation levels recorded in the plot were above the historical average during the reproductive stages while temperatures were very close to historical averages during this time. Considering weather station data for the Glyndon plot during the 2018 growing season, the influence of weather on seed size should be considered normal.

CONCLUSION

This was a preliminary study designed to determine potential plant growth regulators for further study in an effort to reduce seed size for seed production purposes. Based on the results of this study, further research is recommended with the PGR-B chemistry applied at varying rates and application timings. Earlier application of plant growth regulators should be considered as the applications made at the V3 stage were made just prior to flowering. It is recommended that an earlier application would be made in future research to explore the effects of PGR-B application earlier in the vegetative growth stages. Due to the delays seen, additional observations should be considered during the flowering growth stages to determine the impacts of PGR-B on the duration of the flowering process. Potential considerations would be to make an application at the V2 growth stage or to time the application a set number of days after emergence to reduce the variability in application timing. Overall, this study contributes to the existing literature on plant growth regulator use in soybean while also providing preliminary data for further seed production research on plant growth regulators in maturity group 00 and 0 soybean varieties. Based on the findings of this study, it has been determined that select plant growth regulators have the potential to decrease average seed size while still maintaining seed yield.

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Appendix A

Manufacturer Recommendations

Seed Research Equipment Solutions Standard Plot Planter (Seed Research Equipment Solutions, 2005, p 26-31.)

Field Calibration

Field Map Information

The planter can build a field map as you plant, recording seeds per plot, plot time, etc. To do this, the following things need to be set at the beginning of every field to let the planter know its location.

1. In the **Planter Settings** screen,
    a. Enter the **Rows per Plot**. This is to set the rows per plot on the field map.

       Example: On a four-row planter planting two row plots, rows one and two can be recorded as plot one, and rows three and four will be plot two.

2. In the **Plot Settings** screen,
    a. Enter the **Number of Ranges** you are going to plant in a pass, including border ranges.

    b. Enter the **Database Filename** you would like to use. This can be up to eight characters and must end in **.txt**.

3. On the top menu, select **Relocate – Relocate**. This brings you to the **Relocate Range/Plot** screen.
    a. Set the **New Plot Number**, the point in the field you are going to start planting.

    b. Set the **New Range Number**, the point in the field you are going to start planting.

    c. Set the **Range Direction**. This is a toggle button, from increasing (INC) to decreasing (DEC).

    d. Set the **Plot Direction**. This is a toggle button, from increasing (INC) to decreasing (DEC).

    e. Set the **First Turn** direction. This is clockwise (CW) or counter clockwise (CCW).

f. Set the field **Arrangement**. This can be serpentine or straight.

Start Cycle

The SRES Precision Vacuum Planter cycles *before* the alley, in order to have the seeds on the plate in time for the next plot. As a result, the planter must be "primed", or have seed loaded onto the plate, to be ready to start planting at the first plot for every pass. To do this,

1. The operator must be ready for the first pass
2. The vacuum must be at the operating range
3. The seed shaft hydraulics must be engaged
4. The first range's seed must be in the divider/single drops for all rows.
5. The Master On/Off button must show "MASTER ON".
6. Press the star key on the remote box twice within three seconds, OR using the pen, on the top menu, select Start Cycle – Start Cycle.
7. The seed shaft should turn, the slides should cycle, and the divider/single drops should open to load the first plot. There should be a message displayed in bottom center of the screen, "Waiting For First Checkhead".
8. Load the seed in for the next plot, and you are ready to start moving.

Counts per Foot Calibration – *Manual*

The following procedures will calibrate the distance traveled in the computer to the actual distance traveled.

1. Set up the checkhead cable in the field.
2. Turn main power switch on Control Screen Console, Remote Box, and Main Panel to **OFF**.
3. Start tractor.
4. Turn fan on.
5. Turn Main Control Box **ON**.

6. Turn compressor switch **ON**. Turn Alley Wiper Switch **ON**.
7. Turn power switch on computer **ON**.
8. Turn Remote Box **ON**.
9. Double click on the **SRES 2.0** icon to enter the planter program.
10. Select the settings file set up for the field you are about to plant.
11. Position tractor so that it is 24 to 30 inches behind the first button.
12. In the planter run screen, turn the master button **ON**. It should now read "**MASTER ON**".
13. No seed needs to be added for this procedure but you can add seed if you want. Put the checkhead cable into the checkhead.
14. On the top menu, select Start Cycle – Start Cycle (OR: You may instead choose to press the "*" key on the remote box twice within three seconds to activate a Start Cycle). The planter should go through a cleanout and then load the seed for the first plot.
15. In the upper right hand corner of the screen, the distance will be displayed in inches. The distance shown here is the distance traveled between buttons.
16. Drive the tractor through at least five buttons and watch the distance traveled.
17. The distance on the computer should be within 1 to 2% of the actual distance between buttons. If the distance is wrong, go to step 18. If the distance is right, go on to set the **Alley Setback**.
18. On the top menu, go to ***Settings*** – ***Plot Settings***.
19. Change the **Counts per Foot setting**.
20. If the distance on the computer is higher than actual, the counts per foot need to be raised. If the distance on the computer is lower than actual, then the counts per foot need to be lowered. Example: The distance should be 209 and the distance is reading 180. (180 divided by 209 is 0.8612) The counts per foot are currently 32. Multiply 32 by 0.8612. The new counts per foot should be 27.56.

21. Select Close. When asked "Save Settings?" select Save or Save As.

22. Go to step 12.

Counts per Foot Calibration – *Automatic*

The following procedures will automatically calibrate the distance traveled in the computer to the actual distance traveled.

1. Set up the checkhead cable in the field.
2. Turn main power switch on Control Screen Console, Remote Box, and Main Panel to **OFF**.
3. Start tractor.
4. Turn fan on.
5. Turn Main Control Box **ON**.
6. Turn compressor switch **ON**. Turn Alley Wiper Switch **ON**.
7. Turn power switch on computer **ON**.
8. Turn remote box **ON**.
9. Double click on the **SRES 2.0** icon to enter the planter program.
10. Select the settings file set up for the field you are about to plant.
11. On the top menu, select ***Settings*** – ***Calibrate Distance***.
12. Check the box labeled Auto.
13. Select Close.
14. Put the checkhead cable into the checkhead.
15. Drive the tractor through at least 10 or more ranges. At every ten ranges the planter should recalibrate itself from an average of the previous ten ranges.
16. Go on to set the **Alley Setback**.

Alley Setback

This procedure will center the alleyway over the button on the cable. If you are continuing from setting the Counts per Foot, skip to step 12.

1. Set up the checkhead cable in the field.
2. Turn main power switch on Control Screen Console, Remote Screen, and Main Panel to **OFF**.
3. Start tractor.
4. Turn fan on.
5. Turn Main Control Box **ON**.
6. Turn compressor switch **ON**. Turn Alley Wiper Switch **ON**.
7. Turn power switch on computer **ON**.
8. Turn Remote Box **ON**.
9. Double click on the **SRES 2.0** icon to enter the planter program.
10. Select the settings file set up for the field you are about to plant.
11. Put cable into checkhead.
12. Position the tractor so that it is at least 24-30" behind the first button.
13. In the planter run screen, turn the master button **ON**. It should now read "**MASTER ON**".
14. Put seed for the first plot in the single drop or divider.
15. On the top menu, select ***Start Cycle*** – ***Start cycle***, OR press the "*" key on the remote box twice within three seconds to perform a start cycle. The planter should go through a cleanout and then load the seed for the first plot.
16. Put the seed for the second plot in for the single drop or divider.
17. Drive the tractor through at least 5 plots, planting seed.
18. Check to see if the alleyway is centered over the button on the cable.

19. If the alley is centered over the cable, then you are finished setting the Alley Setback. If it is not, then go to step 20.

20. Go to ***Settings*** – ***Planter Settings***.

21. You need to change the **Alley Setback**.

22. If the alley is too far in the direction of travel, then the Alley Setback needs lowered by the amount of distance it is off. If the alleyway is not far enough in the direction of travel, then the Alley Setback needs raised by the amount of distance it is off.

Alley Setback

Button on cable *

Alley Setback 70 Alley Setback 40

--I--I--I--I--*----------I--I→ I--I--I--I---------*--I--I→

23. Select **Close**. When asked "*Save Settings?*" select **Save** or **Save As**.

24. Return to step 11.

Bulk Planting

Bulk Planting is activated by selecting ***Bulk Planting – Disabled*** on the top menu. The "Disabled" will change to show "Enabled", and the message "Bulk Planting" will appear on the bottom center of the run screen.

In bulk planting mode the planter will act like a commercial planter, and the seed shaft will turn in proportion to the speed you are planting. The slides and the divider/single drop will not activate, and there will be no alleys.

If you select **Simulate – Checkhead**, the screen will loop, and the seeds will show constantly, allowing constant monitoring of the seeds planted.

SRES recommends using our bulk hoppers for bulk planting larger areas of fill or strip tests, although this is not required. Bulk hoppers handle a larger volume of seed and faster speeds.

ALMACO Seed Spector LRX Manual (Almaco, 2004, p 4-9.)

WT1, WT2 **Weight 1 and Weight 2**
These selections measure total sample weight at each weigh hopper via a load cell. (Calibrations are generated by the user.)

TM1, TM2 **Temperature 1 and Temperature 2**
These selections measure the temperature at each moisture probe. (Calibrations are generated by the user.)

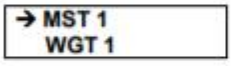
TWT1,TWT2 **Test Weight 1 and Test Weight 2**
These selections measure test weight via a load cell. (Calibrations are generated by the user and the volume of the test weight chamber must be known.)

MOISTURE CALIBRATION

Before beginning, please read the following important moisture calibration procedures.

1. Samples used for calibrating should be uniform and homogeneous. Do not mix samples of different moistures to obtain intermediate samples. Large chunks of cob or other trash will cause readings to shift.
2. Samples should enter the moisture-sampling chamber the same way they do when the combine is harvesting. For calibrating, the samples are poured into the holding hopper and then dumped into the weigh hopper and moisture chamber from the holding hopper. This is the same as if the combine were harvesting plots.
3. The combine should be running the same as during harvest. That is, the shakers, header & cylinder operating at the same RPM as they do when the crop is being harvested. The machine vibration causes the grain to settle in the moisture-sampling chamber, so for the calibration to be accurate the conditions must simulate harvest as close as possible. Also the sample needs to set in the moisture-sampling chamber for the same amount of time as it normally would during the harvest procedure and be subject to the same amount of vibration, prior to the calibration value being entered in the Seed Spector.
4. Care should be used in the handling of higher moisture samples. Exposure to the air will cause them to loose moisture. So minimize the handling of the samples, and recheck the moistures after the calibration process is completed to make sure that they did not change too much.

To calibrate the moisture, use the down arrow to select '**Calibrate**' from the main menu. If starting from the run screen, press the ESC key to display the main menu. Press the EXE key and the calibration data types appear.



With the arrow highlighting MST 1, press the EXE key to display the calibration menu.

```
→ Initialize
  Generate
```

A new moisture calibration requires the user to select and execute '**Initialize**' after which the data type had been selected. The Initialize feature erases the old calibration from the SS-LRX memory. If the initialization is not done, the new moisture points will be added to the existing calibration causing inaccuracies. Highlight the Initialize option and press the EXE key.

```
→ Init MST 1 ?
  NO
```

Press the down arrow key to move the selection arrow to '**YES**' and press the EXE key. After 'MST 1' has been initialized, the SS-LRX returns to the calibration menu screen.

```
  NO
→ YES
```

The SS-LRX is ready to be calibrated with the known samples. At the calibration menu, highlight the '**Generate**' and press the EXE key to display the calibration entry screen. The 'MST 1' indicates the data being calibrated and the 'New 1' represents the point of the calibration.

```
MST 1    New 1
[   0.00]  0000
```

To calibrate the moisture reading the user will need to use several samples of grain, each with a known moisture percentage. The moisture contents of these samples should be determined using the ASAE dry down method or a calibrated moisture meter such as the Dickey-john GAC 2000. To develop the most accurate curve inside the Seed Spector LRX, it's recommended that the lowest moisture sample be inputted first. The next sample needs the highest moisture, which should represent the highest moistures measured in the field during harvest. After these two samples have been entered, the remaining grain samples can be input at random. A minimum of two samples is required for a valid calibration. Three or four samples shoud be used, if a wide range of moistures is being harvested. Adjacent samples should be no closer than 3-4% in absolute moisture.

Sample Entry
To calibrate the moisture, enter the moisture values in the left side of the display. After the proper moisture value has been entered, press the right arrow key and move the brackets around the 0000 value. Place the grain from the holding hopper into the weigh bucket containing the probe.

```
MST 1    New 1
  12.5   [ 0000 ]
```

Press the right arrow again and the raw data will appear on the right side of the display. The value is not a real-time reading, so the right arrow button needs to be pressed again to verify a stable value being displayed.

```
MST 1    New 1
  12.5   [ 0548 ]
```

Press the EXE key to accept the stable raw data value. After the value has been accepted, the SS-LRX will be advance to the next moisture sample. The 'MST 1' indicates the same data curve is being calibrated and the 'New 2' represents the second point of the calibration.

```
MST 1    New 2
[  0.00]   0000
```

Repeat this procedure with all of the grain samples. The accuracy of the curve will be dependent upon the number of samples and the accuracy of the known sample moisture. When the calibration process is complete, press ESC key three times to return to the Main Menu.

Manual Entry
To calibrate the moisture with known values, enter the moisture on the left side of the display. After the proper moisture value has been entered, press the right arrow key and move the brackets around the 0000 value.

```
MST 1    New 1
  12.5   [ 0000 ]
```

Using the keypad, manually enter the known raw moisture reading.

```
MST 1    New 1
  12.5   [ 0548 ]
```

After the value has been entered, press the EXE key to accept the setting. Do not press the right arrow because the entered value will be overwritten with the real-time reading. After the value has been accepted, the SS-LRX will be advance to the next moisture sample entry. The 'MST 1' indicates the same data curve is being calibrated and the 'New 2' represents the second point of the calibration.

MST 1	**New 2**
[0.00]	**0000**

Repeat this procedure with all of the grain samples. The accuracy of the curve will be dependent upon the number of samples and the accuracy of the known sample moisture. When the calibration process is complete, press ESC key three times to return to the Main Menu.

WEIGHT CALIBRATION

Before beginning, please read the following important weight calibration procedures.

1. The suspension of the weigh bucket should be checked for friction. Any tie rod ends should rotate freely. All tie rods need to be installed to not influence the weight readings. The rods should be in the horizontal position. To check for friction, press down on the weigh hopper and release. With the weight displayed on the Seed Spector, observe the stabilized weight reading. Then lift up on the weigh hopper and release, observe the stabilized Seed Spector reading. A variation in the two readings indicates there is friction in the hopper system. Repeat the procedure several times to verify. Correct any problems with the hopper suspension before calibrating the weight.
2. It is recommended that the engine of the combine not be running when the weight is being calibrated. Or if it is necessary to run the engine, then none of the combine mechanisms should be engaged to cause excess vibration during the weight calibration. The calibration routine in the Seed Spector is designed to reject entry of data if the weight readings are changing too much due to vibration. So it is best to calibrate with the engine off.

To calibrate the weight, use the down arrow to select '**Calibrate**' from the main menu. Press the EXE key and the calibration data types appear.

MST 1
→ WGT 1

With the arrow highlighting WGT 1, press the EXE key to display the calibration menu.

→ **Initialize**
Generate

A new weight calibration requires the user to select and execute '**Initialize**' after the data type has been selected. The Initialize feature erases the old calibration from the SS-LRX memory. If the initialization is not done, the new weight points will be added to the existing calibration causing inaccuracies. Highlight the Initialize option and press the EXE key.

→ **Init WGT 1 ?**
NO

Press the down arrow key to move the selection arrow to '**YES**' and press the EXE key. After 'WGT 1' has been initialized, the SS-LRX returns to the calibration menu screen.

NO
→ **YES**

The SS-LRX is ready to be calibrated with the known samples. At the calibration menu, highlight the '**Generate**' and press the EXE key to display the calibration entry screen. The 'WGT 1' indicates the data being calibrated and the 'New 1' represents the point of the calibration.

WGT 1	**New 1**
[0.00]	**0000**

Only two known weights are necessary for calibrating a load cell. The empty bucket should provide the first calibration point. Enter the '0.0' in the left side of the display for the empty bucket weight. Press the right arrow key and move the brackets around the 0000 value.

WGT 1	**New 1**
0.0	**[0000]**

Press the right arrow again and the raw data will appear on the right side of the display. The value is not a real-time reading, so the right arrow button needs to be pressed again to verify a stable value being displayed.

WGT 1	**New 1**
0.0	**[1234]**

Press the EXE key to accept the stable raw data value. After the value has been accepted, the SS-LRX will be advance to the next weight value. The 'WGT 1' indicates the same data curve is being calibrated and the 'New 2' represents the second point of the calibration.

WGT 1	New 2
[0.00]	0000

Enter the value of the second weight in the left side of the display and press the right arrow key. Position the known weight in the bucket, so the force of the weight is directly over the load cell or beam. If the weight isn't position on the bucket properly, calibration inaccuracies can occur from bucket tension.

WGT 1	New 2
10.02	[0000]

Press the right arrow again and the raw data will appear on the right side of the display. The value is not a real-time reading, so the right arrow button needs to be pressed again to verify a stable value being displayed.

WGT 1	New 2
10.02	[2987]

Press the EXE key to accept the stable raw data value. When the calibration process is complete, press ESC key three times to return to the Main Menu.

MANUAL ENTRY

Use the same procedure described for the moisture calibration to manually enter the weight calibration. If you plan to use a manual calibration entry, you must write down the values while you are calibrating, view the file after you are done calibrating, or have an electronic copy of the file.

TEST WEIGHT CALIBRATION

'**TWT1**' and '**TWT2**' are the designations for the test weight load cells. Calibrating the test weight is comparable to calibrating the weight channels. Refer to the **WEIGHT CALIBRATION** section and select '**TWT1**' and '**TWT2**' appropriately at the Data Type Menu.

Appendix B

Permission granted for photo and weather station data

From: MADDEN, SCOTT [redacted]
Sent: Monday, March 04, 2019 2:37 PM
To: WETTERLING, MATTHEW G [redacted] RUTHERFORD, AMY L [redacted]
Subject: RE: Approval for use of company pictures for ISU MS Agronomy program

I approve. Nothing there noting any company or varietal information.

From: WETTERLING, MATTHEW G [redacted]
Sent: Monday, March 04, 2019 2:15 PM
To: RUTHERFORD, AMY L [redacted] MADDEN, SCOTT [redacted]
Subject: Approval for use of company pictures for ISU MS Agronomy program

Amy and Scott,
I just sent you a OneDrive link for the 8 pictures I would like to use in my paper/presentation. I have listed the photos below.

01_WatchDog Weather Station
02_Chemical Application
03_Plot Layout
04_R5 Pod Count
05_R8 Pod Count
06_June Field Photo
07_Harvest Bag
08_Emergence

Please respond to this email for approval.

Thanks,
Matt Wetterling

Appendix C
Additional Charts

	May 23-31	June	July	August	September	October 1-22
Average High Temperature °F	73.2	77	82	81	71	59
Average Low Temperature °F	49.3	55	59	57	49	37
Average Precipitation in Inches	.92	3.51	2.88	2.52	2.18	1.41

Table 11. Average Climate for Moorhead, MN (Weather Underground, 2019).

	May 23-31	June	July	August	September	October 1-22
Average High Temperature °F	87.77	81.35	82.13	79.97	70.36	47.75
Average Low Temperature °F	62.8	59.36	58.91	56.5	47.35	30.98
Average Precipitation in Inches	0.14	5.32	3.87	3.23	1.04	1.48

Table 12. Weather Station Data from Glyndon, MN Plot